REMARKS

Claims 1 through 6 and 17 through 25 are pending in the application.

Claims 1 and 2 have been amended to recite positive methods steps in conformance with United States practice. Support for this amendment can be found in the Application-as-filed.

Claim 1 has also been amended to recite the unit "U per ml" in more conventional US format, i.e. as a discrete phrase without intervening text. Support for this amendment can be found in the Application-as-filed, for example in Claim 1 as-submitted and on Page 4, lines 19 through 21.

Claim 1 has further been amended to positively recite that the inventive reaction medium includes the test medium and ADAMTS-13-free, urea treated von Willebrand factor, and that platelets are added to the reaction medium subsequent to its incubation. Support for this amendment can be found in the Application-as-filed.

Claims 1 and 2 have additionally been amended to recite that the test medium initially includes an unquantified level of ADAMTS-13, which is subsequently quantified based on either platelet aggregation (as reflected in Claim 1) or the dissociation of platelet aggregates (as reflected in Claim 2) within the incubated reaction medium. Support for this amendment can be found in the Application-as-filed.

Claim 2 has also been amended to positively recite the step of initially aggregating platelets by incubating the platelets with ADAMTS-13-free von Willebrand factor (VWF). Support for this amendment can be found in the Application-as-filed.

Claims 17 and 24 have been amended to complete the record for examination by reciting further advantageous alternative embodiments in which the test medium is blood plasma, blood serum, saliva, cerebrospinal fluid, cell culture supernatant. Support for this amendment can be found in the Application-as-filed, for example on Page 4, lines 25 through 29.

Claim 22 has been canceled.

Reexamination and reconsideration of this application, withdrawal of all rejections, and formal notification of the allowability of the pending claims are earnestly solicited in light of the remarks which follow.

Section 112 Rejection

Claim 1 stands rejected over the phrase "0.5 to 5 U." Claim 1 has been amended to recite the unit "U per ml" as a discrete phrase, i.e. without intervening text. As noted above, support for this non-narrowing amendment can be found in the Application as filed, including Claim 1 as-filed.

Applicant further respectfully submits that one skilled in the art readily understands that pooled normal plasma is deemed to contain 100% VWF and that one U per ml is defined as 100% VWF. Applicant further respectfully submits Exhibit 1: *Detection of von Willebrand Disorder* from American Journal of Clinical Pathology, evidencing the equivalence of 1 U per ml with 100% VWF on Page 3, first partial paragraph, lines 5 through 6. Accordingly, Applicant respectfully requests withdrawal of this rejection.

Claims 1 and 2 stand further rejected over a purported failure to recite positive steps. Without addressing the merits of the rejection and solely to advance prosecution of the case, Claims 1 and 2 have been amended to recite positive steps, in conformance with more traditional method practice. Accordingly, Applicant respectfully requests withdrawal of this rejection.

The Claimed Invention is Patentable
in Light of the Art of Record

Claims 1 through 6 and 17 through 25 stand rejected under 35 U.S.C. 102(b) as being anticipated by Furlan et al. (Blood 1996;87:4223-4234).

It may be useful to briefly consider the invention before addressing the merits of the rejection.

Von Willebrand Factor (VWF) is a glycoprotein circulating in plasma which mediates initial platelet adhesion. Endothelial cells release von Willebrand Factor in the form of large multimers which, in normal plasma, are cleaved by the combined action of a reductase and a metalloprotease. The metalloprotease has recently been identified as a new member of the ADAMTS family, and designated ADAMTS-13.

Heretofore, methods of investigating ADAMTS-13 within plasma have generally required highly specialized equipment and expertise. For example, conventional electrophoretic and immunoblotting methods, such as described in Furlan, can only be carried out in specialized research laboratories. Furthermore, electrophoresis and immunoblotting are generally used to fractionate and visualize VWF multimers.

Rather than merely separate multimers, Applicant has found methods by which ADAMTS-13 activity can be quantified in a reliable and timely manner in any routine clinical coagulation laboratory. In particular, Applicant has discovered that ADAMTS-13 activity can be quantified based on either (i) platelet aggregation or, conversely, (ii) by the dissociation of platelet aggregates by combininga test medium comprising an unquantified ADAMTS-13 activity with advantageous quantities of ADAMTS-13-free VWF **and platelets.**

In one embodiment of the invention (claim 1) the test medium is incubated with VWF first, then platelets are added and the ADAMTS-13 activity is determined by quantifying the aggregation of the platelets. In another, alternative embodiment (claim 2) VWF is incubated with platelets first leading to platelet aggregates, then the test medium is added and the ADAMTS-13 activity is determined by quantifying the dissociation of the platelet aggregates.

Accordingly, claim1 is directed to diagnostic methods for determining the VWF-cleaving activity of ADAMTS-13 in a test medium in which an ADAMTS-13-free von Willebrand factor (VWF) is incubated with urea. A test medium, such as blood plasma, having an unquantified ADAMTS-13 activity level is provided, and from 0.5 to 5 U per ml of ADAMTS-13-free von Willebrand factor is added to the test medium, thereby forming the reaction medium. The reaction medium is then incubated, and platelets are added to the incubated reaction medium. The ADAMTS-13 activity is subsequently quantified based on the VWF-mediated aggregation of the platelets within the reaction medium.

Claim 2 is directed to diagnostic methods for determining the VWF-cleaving activity of ADAMTS-13 in a test medium in which platelets are initially aggregated by incubation with an ADAMTS-13-free von Willebrand factor (VWF). The test medium is then added to the aggregated platelets and the ADAMTS-13 activity of the test medium is subsequently quantified based on the dissociation of the platelet aggregates.

The cited reference does not teach or suggest the claimed invention.

In contrast to the claimed methods,Furlan merely attempts to determine (i) where the protease responsible for cleaving VWF resides and (ii) to characterize the protease he recovers from the various materials. More specifically, Furlan analyses different materials, like plasma, serum, cryoprecipitate-free plasma, defibrinated plasma 15-fold concentrated cryoprecipitate and lysed platelets to determine in which material the protease is present (see Page 4225-4226

spanning paragraph and Page 4232, left hand column, first paragraph, noting high vWF cleaving acitivty of platelet poor plasma, indicating the protease did not originate from platelets).

Furlan subjects the recovered protease to a number of enzyme inhibitors in attempt to determine its identity, ultimately concluding that further studies are required. (Page 4229, right hand column, first partial paragraph, and Page 4232, right hand column, first full paragraph, "further studies are required to elucidate the identity of the plasma protease" and Page 4224, left hand column, noting that the proteolytic activity is associated with a HMW protein that is "different" from known proteases). Furlan specifically notes on numerous occasions that the protease was not a serine protease as it "was not affected" by DFP. (Page 4225, left hand column, second full paragraph, second sentence, noting the results of preliminary experiments and Page 4229, right hand column, first partial paragraph, noting "[t]here was ... no inhibition by serine protease inhibitors DFP" and Page 4232, left hand column, stating "the enzyme is no serine protease because it was not affected by any of the serine protease inhibitors used.")

Furlan subjected the protease-treated-VWF to conventional electrophoresis and immunoblotting to determine the resulting VWF multimer pattern. (Page 4224, 1st Col., 4th full paragraph through 2nd Col. 4th full paragraph and Page 4225, 1st Col. 2nd full paragraph).

Applicant respectfully submits that Furlan does not teach or suggest the claimed invention.

More specifically, Furlan does not teach either the recited addition of platelets or subsequent platelet aggregation (or dissociation of platelet aggregates) and thus can not anticipate the claimed invention.

Furlan likewise fails to even suggest the recited use of platelets and platelet aggregation (or dissociation of platelet aggregates), much less methods in which platelet aggregation (or dissociation of platelet aggregates) are used to quantify ADAMTS-13 activity.

Applicant respectfully submits that the only platelets used in conjunction with Furlan's study were lysed platelets (i.e. burst platelets) which were used as a potential protease-source. Furlan thus most certainly does not teach or suggest the recited use of platelets as a diagnostic tool. The only analysis performed by Furlan is based on fractionation, i.e. by electrophoresis and subsequently immunoblotting.

Applicant respectfully submits that the Office Action incorrectly appears to indicate that Furlan somehow correlates platelet aggregation using electrophoresis and immunoblotting. Applicant respectfully makes of record that their inventive use of the recited platelet aggregation (claim 1) or aggregated-platelet-dissociation (claim 2) as a means to quantify ADAMTS-13 activity is an altogether different analytical methodology than Furlan's electrophoresis and immunoblotting techniques, which are based on VWF fragment analysis

Applicant further respectfully submits that there would have been no motivation for Furlan to have quantified the ADAMTS-13 activity of a test medium using platelet aggregation or dissociation.

In fact, Applicant respectfully submits that there would have been no motivation to have looked to Furlan. Furlan is an academic study that attempts to find the source of the protease that cleaves VWF into a particular multimer pattern found in vivo and to subsequently characterize that protease. Furlan merely subjects the resulting VWF multimer to fragment analysis using electrophoresis and immunoblotting,, requiring expensive, complex equipment.

In contrast, the instant invention is directed to methodologies incorporating platelets as a diagnostic means to determine the ADAMTS-13 activity of test material. Surprisingly, the claimed methodologies quantify the ADAMTS-13 level within the test material using methods

readily available with routine clinical coagulation laboratories, i.e. platelet aggregation techniques.

Accordingly, Applicant respectfully submits that Claims 1 and 2 (and claims dependent thereon) are patentable in light of Furlan.

Applicant additionally respectfully submits that Claims 3 and 21, further reciting the presence of ristocetin, are likewise patentable in light of Furlan.

Applicant more particularly respectfully makes of record that there would have been no motivation to have utilized ristocetin in conjunction with Furlan, in contrast to the opinion urged within the Office Action. Ristocetin-based platelet aggregation is performed using drawn blood samples to diagnose von Willebrand disease. As noted above, Furlan is a purely academic study directed to finding the source and character of a protease which cleaves VWF into an expected multimer pattern. In the course of his investigation, Furlan merely separates the resulting VWF multimers using conventional electrophoresis and immunoblotting. As noted above, there would have been no motivation for Furlan to used platelet aggregation (or dissociation of platelet aggregates), much less to have used ristocetin.

Applicant further respectfully submits that there would have been no motivation to combine ristocetin with Furlan, as there would have been no expectation of success. Applicant respectfully submits that "obvious to try" is not the standard for patentability.

Applicant also respectfully submits Claims 6 and 23, further reciting the presence of a serine protease inhibitor, are likewise patentable in light of Furlan. Applicant respectfully submits that Furlan actually teaches away from such advantageous embodiments by instead

indicating on multiple occasions that such inhibitors are ineffective in combination with his protease.

Accordingly, Applicant respectfully submits that the claimed invention is patentable in light of Furlan, considered either alone or in combination with the art of record.

Consideration of Previously Submitted Information Disclosure Statement

It is noted that an initialed copy of the PTO/SB/08A and B that were submitted with Applicant's Information Disclosure Statement filed November 10, 2006 has not been returned to Applicant's representative with the Office Action. Accordingly, it is requested that an initialed copy of the PTO/SB/08A and B form be forwarded to the undersigned with the next communication from the PTO. In order to facilitate review of the references by the Examiner, a copy of the Information Disclosure Statement and the PTO/SB/08A and B forms are attached hereto. Copies of the cited references were provided at the time of filing the original Information Disclosure Statement, and, therefore, no additional copies of the references are submitted herewith. Applicant's Representative will be pleased to provide additional copies of the references upon the Examiner's request if it proves difficult to locate the original references.

CONCLUSION

It is respectfully submitted that Applicant has made a significant and important contribution to the art, which is neither disclosed nor suggested in the art. It is believed that all of pending Claims 1 through 6 and 17 through 25 are now in condition for immediate allowance. It is requested that the Examiner telephone the undersigned if any questions remain to expedite examination of this application.

It is not believed that extensions of time or fees are required, beyond those which may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time and/or fees are necessary to allow consideration of this paper, such

extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required is hereby authorized to be charged to Deposit Account No. 50-2193.

Respectfully submitted,

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CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office at facsimile number (571) 273-8300 on May 16, 2007.

Claire Wygand

Claire Wygand

Exhibit I

Detection of von Willebrand Disorder
from American Journal of Clinical Pathology

Materials and Methods

Sample Testing

All vWD samples were derived from patients diagnosed as having vWD using standard criteria[2,3] and obtained with informed consent. All vWD samples were assessed for factor VIII, vWF:Ag, vWF:CBA, and vWF:RCof.[8,12,13,22] Further subtyping analysis was undertaken whenever possible in consultation with the referring clinician and using vWF:multimer analysis and/or ristocetin-induced platelet aggregation testing as appropriate.[8,12,13,22] In some cases, patients were further assessed using DNA analysis.[29,30] Evidence of vWF discordance (ie, qualitative or functional vWF defect) was noted whenever relevant.[8,12,13,22] In addition to vWD plasma, a number of normal samples were collected and used for comparative studies, as well as for generation of a pooled normal plasma (typically >60 persons). All individual plasma samples were prepared following collection into standard buffered sodium citrate tubes (0.105-mol/L concentration of citrate: citrate/blood, 1:9) and centrifuged (1,200*g*; 16 minutes) to isolate plasma. Samples were frozen in aliquots at -80°C until required for comparative studies.

Laboratory Studies

Methods for standard assay procedures (eg, vWF:Ag, vWF:CBA, vWF:RCof) have been published.[8,12,13,22] vWF:RCof was performed using a standard agglutination assay with fixed platelets, whereas vWF:Ag and vWF:CBA were performed using ELISA.[8,12,13,22] Commercial ELISA kit procedures comprised similar standard sandwich ELISA techniques. All manufacturers agreed to provide 2 or more kits from a single current lot of their product for evaluation. The present study coevaluated the following commercial options, all of which were used according to manufacturer's instructions:
1. vWF:activity ELISA (functional anti-vWF MAB-based; Shield Diagnostics; supplied by Dade-Behring, Australia); the current version 1 (Mark II, the newly modified procedure, product code FvWF200) and the current version 2 (Mark I, the original procedure, product code FvWF100) kits were tested. Both are similar but differ in terms of the assay detection system. In the Mark I kit, a horseradish peroxidase (HRP)-conjugated rabbit anti-vWF is used; in the Mark II kit, HRP-conjugated MAB anti-vWF is used.

2. vWF:CBA test kits from (a) IMMUNO AG (IMMUNOZYM vWF:CBA, distributed by PROGEN IMMUNO-Diagnostika, Heidelberg, Germany) and (b) Gradipore (Collagen Binding Assay, product code CBAE-1, Gradipore, Sydney, Australia).

The sandwich ELISA process initially involves coating plates with material capable of binding vWF (ie, collagen or antibody = first layer, which enables vWF capture). After incubation and washing, plasma (containing vWF) is added, and vWF subsequently is bound to plates (the second layer, ie, vWF captured by first layer). After further incubation and washing, a third layer is introduced, this time comprising the antibody to vWF, and together with subsequent additional layers forming the vWF detection system. Thus, in the vWF:Ag procedure, plates are coated with rabbit anti-vWF antisera (R-anti-vWF; DAKO, Sydney, Australia), followed by sequential washing and incubation steps using plasma (source of vWF), HRP-labeled R-anti-vWF (DAKO), and color generation using tetramethyl benzidine hydrochloride as HRP-substrate.[8,12,13,22] The in-house vWF:CBA, the commercial vWF:CBA assays, and the commercial MAB-based kit methods are technically similar but respectively use collagen or an MAB against vWF as the first layer/vWF-capture process instead of R-anti-vWF. This is summarized in Table 1.

Unless otherwise stated, conditions for all in-house ELISAs (eg, sample and reagent incubation times, blocking buffer, washing steps, washing buffer, plasma dilutions, HRP-substrate, and ELISA plates) were essentially identical and compareble to those used in standard vWF:Ag and vWF:CBA assays at the author's laboratory.[8,12,13,22] For the vWF:Ag assay, rabbit antibody to vWF (Dakopatts, Sydney, Australia) is diluted in a 0.1-mol/L concentration of $NaHCO_3$ buffer (pH 8.3) for coating ELISA plates (EIA plates, ICN, Sydney, Australia; 200 µL per well; plate left covered overnight at 4°C in a wet-box before use on the day of assay). For vWF:CBA, the reference method involved using Nycomed-HORM collagen (Nycomed Arzneimittel, Ismaning, Germany; 1 mg collagen per milliliter of stock) diluted to a final concentration of 50 µg/mL using HORM buffer containing 0.1% sodium azide and left to coat onto ELISA plates (200 µL of collagen solution per well) for 4 days at room temperature in a wet-box. The HORM collagen source is derived from equine tendon and is described by the manufacturer as a type I/type III collagen mixture (95%/5%, respectively). As an alternative to the Westmead Hospital Laboratory reference vWF:CBA method, type III collagen (derived from human placenta; Sigma, Sydney, Australia; code number C4407; lot number 65H39041) also was used for comparative studies as previously described[22] (NB: this type III collagen is believed to be similar to that used in the IMMUNO CBA kit).

On the day of use, all in-house-derived assay plates were washed 3 times with wash buffer and blocked for 1 hour using 5% bovine serum albumin. Thereafter, plates were treated according to a standard vWF ELISA procedure (see elsewhere for additional details).[8,12,13,22]Unless otherwise stated, patient plasma samples were tested in triplicate for each experiment at a final dilution of 1:100. Pooled normal plasma (deemed to contain 100% vWF) was used to generate comparative calibration curves for each in-house assay. In addition, control plasma samples (in-house [including cryosupernatant] and commercial [eg, assayed reference plasma, SARP, Helena, Mulgrave, Australia]) were used in each experiment.

All commercial kits were used according to the manufacturer's instructions. To more fairly compare test results from all assays,

however, and as standardly performed for the Westmead Hospital Laboratory in-house method, samples were tested in triplicate rather than in duplicate as suggested in the commercial product inserts. Calibration curves were generated using kit-provided material but normalized if required to the percentage of vWF (ie, where 1 U/mL is defined as equivalent to 100% vWF). Kit-provided controls also were run with each assay, in addition to other controls (ie, pooled normal plasma, SARP, cryosupernatant). Data derived for all tested controls for all assays were consistently within stated or expected limits.

In general, multiple assays were performed on the same or sequential days using freshly thawed or reconstituted samples, and most tested samples were tested in all assays (ie, same sample sets used for most assays). In addition, some samples were retested in the same assay type on different days to help assess reproducibility. However, because of variations to the recommended construction of calibration curves for the commercial kits and the recommended use of commercial controls, additional incorporation of other controls, and limited availability of the commercial kits, not all samples were tested in all assays. Accordingly, as indicated in the "Results" section, sample numbers tested between assays on occasion varied slightly. This is not expected to unduly influence interpretation of results.

Statistical Analysis

All data were analyzed using GraphPad Prism 2.0C (GraphPad Software Inc, San Diego, CA) for the Macintosh. Data obtained for samples retested in the present study also were sometimes compared with historic data obtained on samples before storage (ie, to identify potential variance).

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